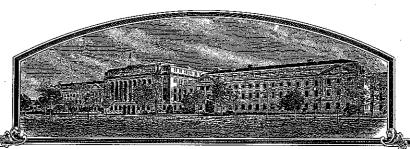
No.



200300078

HHE UNITED SHAMES OF AMERICA

TO ALL TO WHOM THESE; PRESENTS SHATE COME;

Haragon Seed, Inc.

MICIONS, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED DISTINCT VARIETY OF SEXUALLY REPRODUCED, OR TUBER PROPAGATED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID, APPLICANT(S) FOR THE TERM OF TWENTY YEARS FROM THE DATE OF THIS GRANTS SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE IGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR SKI ING IT, OR EXPORTING IT, OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE PURPOSE OR CONDITIONING IT FOR PROPAGATION, OR STOCKING IT FOR ANY OF THE ABOVE OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT BY THE PLANT VARIETY PROTECTION ACT. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

LETTUCE

'Tehama'

In Jestimonn Mucrost, I have hereunto set my hand and caused the seal of the Hunt Hariety Frotestion Office to be affixed at the City of Washington, D.C. this seventeenth day of April, in the year two thousand and six.

Plant Variety Protection Office Agricultural Marketing Scruice

U.S. DEPARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE SCIENCE AND TECHNOLOGY - PLANT VARIETY PROTECTION OFFICE

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE

The following statements are made in accordance with the Privacy Act of 1974 (5 U.S.C. 552a) and the Paperwork Reduction Act (PRA) of 1995.

Application is required in order to determine if a plant variety protection certificate is to be issued (7 U.S.C. 2421). Information is held confidential until certificate is issued (7 U.S.C. 2426).

(Instructions and information	collection burden stateme	ent on reverse	e) (1 0.0.0.2421)	. microstich is neid Contidential	unui ceruncate	is issued (7 U.S.C. 2426).
1. NAME OF OWNER				2. TEMPORARY DESIGNA EXPERIMENTAL NAME	TION OR	3. VARIETY NAME
Para	Paragon Seed, Inc.			G54 - 27	1	Tehama
4. ADDRESS (Street and No., or R.F.D. No.	, City, State, and ZIP Code, and Co	ountry)		5. TELEPHONE (include are	574	FOR OFFICIAL USE ONLY
507	507 Abbott Street			831-753-210	100	PVPO NUMBER
	Salinas, California 93901			831-/33-210		
3811	mas, Carrorn	14 95	901	6. FAX (include area code)		0030007
				831-753-147		ILING DATE
7. IF THE OWNER NAMED IS NOT A "PERS ORGANIZATION (corporation, partnership	SON", GIVE FORM OF	8. IF INCO	ORPORATED, GIVE OF INCORPORATION	9. DATE OF INCORPORATI	ON	
Corpor	_		ifornia	March 7,19	94	Jan. 15, 2003
10. NAME AND ADDRESS OF OWNER REP	RESENTATIVE(S) TO SERVE IN	THIS APPLICATION	ON. (First person listed will re	eceive all papers)		FILING AND EXAMINATION FEES:
	Heintzberger	-			F	\$ 2705.00
P.O. B	ox 1906				S HERS	
Salina	s, California	9390	02-1906		RHCH	DATE 1-15-2003
					E	CERTIFICATION FEE:
					Ë	\$
· · · · · · · · · · · · · · · · · · ·					· _	DATE
11. TELEPHONE (Include area code)	12. FAX (include area code)		13. E-MAIL		1	(IND (Common Name)
831-753-2100	831-753-1	470	lettuceseed	1@aol.com	Let	tuce
15. GENUS AND SPECIES NAME OF CROP		ļ.	16. FAMILY NAME (Botanio	•	17. IS THE HYBRID	VARIETY A FIRST GENERATION ?
Lactuca sa	tiva L.		Composit	ae	· · □	YES X NO
 CHECK APPROPRIATE BOX FOR EACH reverse) 	ATTACHMENT SUBMITTED (Foils	ow instructions or	n 19. DOES THE C	OWNER SPECIFY THAT SEED (SEED? See Section 83(a) of	OF THIS VARIS the Plant Vari	ETY BE SOLD AS A CLASS OF ety Protection Act)
 a.	•			YES (If "yes", answer items 20 and 21 below)	<u> X</u>	NO (If "no", go to item 22)
c. 🔀 Exhibit C. Objective Description			20. DOES THE C	OWNER SPECIFY THAT SEED (OF THIS	☐ YES ☑ NO
d. X Exhibit D. Additional Description			i i	CHICLASSES? FOUND		REGISTERED CERTIFIED
e. X Exhibit E. Statement of the Bas f. X Voucher Sample (2,500 viable u	intreated seeds or, for tuber propag	gated varieties,	24 DOED THE	NAMED ORGANIA THAT OFFICE		
repository)	ill be deposited and maintained in a	•		DWNER SPECIFY THAT SEED OLIMITED AS TO NUMBER OF		
g. Filing and Examination Fee (\$2, States" (Mail to the Plant Variet)	705), made payable to "Treasurer of Protection Office)	of the United	IF YES, SPEI NUMBER 1,2	' - ' '	ON []	REGISTERED CERTIFIED
				explanation is necessary, please	use the space	indicated on the reverse.)
22. HAS THE VARIETY (INCLUDING ANY HA FROM THIS VARIETY BEEN SOLD, DISP OTHER COUNTRIES?	RVESTED MATERIAL) OR A HYB. OSED OF, TRANSFERRED, OR U	RID PRODUCED ISED IN THE U. S	23. IS THE VARI PROPERTY	ETY OR ANY COMPONENT OF RIGHT <i>(PLANT BREEDER'S RI</i>	THE VARIETY SHT OR PATE	PROTECTED BY INTELLECTUAL NT)?
X YES	□ №		•	YES	Ε	NO
IF YES, YOU MUST PROVIDE THE DATE FOR EACH COUNTRY AND THE CIRCUI	OF FIRST SALE, DISPOSITION, MSTANCES. (Please use space in	TRANSFER, OR dicated on revers	USE IF YES, PLEA se.) REFERENCE	SE GIVE COUNTRY, DATE OF NUMBER. (Please use space I	FILING OR IS: ndicated on rev	SUANCE AND ASSIGNED erse.)
24. The owners declare that a viable sample of for a tuber propagated variety a tissue cult.	f basic seed of the variety will be fu tre will be deposited in a public rep	mished with appl cository and main	lication and will be replenishentained for the	ed upon request in accordance w	ith such regula	tions as may be applicable, or
The undersigned owner(s) is(are) the owner and is entitled to protection under the provi-	er of this sexually reproduced or lub sions of Section 42 of the Plant Va	er propagated pi riety Protection A	ant variety, and believe(s) th	at the variety is new, distinct, un	iform, and stab	le as required in Section 42,
Owner(s) is(are) informed that false represe		•••				
SIGNATURE OF OWNER WWW.	Jan & Seg		SIGNATURE OF	OWNER		
NAME (Please print or type)	/	· · · · · · · · · · · · · · · · · · ·	NAME (Please pri	int or type)		
Vic	tor Heintzber	ger				
CAPACITY OR TITLE Presid	ent	01/10/	03 CAPACITY OR TI	TLE		DATE

Paragon Seed, Inc.

 $_{\rm PVP\,\#} \, \underline{\ \ 2\,0\,0\,3\,0\,0\,0\,7\,8}$

Lettuce Application

Exp. G54-273

TEHAMA

22.) Date of first sale Tehama: Salinas, California, USA

Delivery date: August 22, 2002

Invoice date: September 20, 2002

The pollen parent selected for cross pollination in the development of Tehama was the variety Ventana.
Ventana is a development of Paragon Seed, Inc.
The Application for Ventana Plant Variety Protection was sent to the United States Department of Agriculture on March 15, 1999.
The Application Date for Ventana is March 22, 1999.
The Application Number for Ventana is 9900223

Origin and Breeding History Tehama

The objective of this lettuce development project was to create a new green leaf lettuce variety with Corky Root resistance, bolt tolerance, tipburn resistance, and a darker green color than North Star or Ventana.

To achieve this goal, the Paragon Seed, Inc. breeding line 171669-5-4 was selected as the pollen parent. This breeding line was the numbered line that previously became the variety Ventana. Ventana was developed for its bolt tolerance, tipburn resistance, and Corky Root resistance. Ventana was the first green leaf lettuce with the (cor) gene for Corky Root resistance

A single plant selection from the cultivar Two Star, designated 'DKGrnlf21fr2' was selected as the female (receptor) plant. This Two Star selection exhibited the dark green color desired in our new lettuce, but was susceptible to Corky Root Rot. This selection was part of a "leaf-breeding" program acquired by Paragon Seed, Inc. from Eagle Research and Development, Inc. of Salinas, California in 1996.

In July of 1997, a hand pollinated cross was effectuated between Two Star \mathbf{x} Ventana near Corcoran, California. F_1 seed was harvested in August 1997 and designated G54. A second cross was made with Two Star \mathbf{x} 171669-5-2, a sister line to 5-4 which was designated G52.

All crosses were made using the technique outlined by Ryder and Johnson in "Mist Depollination of Lettuce Flowers", published in HortScience, Vol. 9 (6), 1974.

Seed color of both parental lines was black; seed color of the F_1 seed was black.

In November, 1997, seedlings of each cross were started in a petrie dish and transferred to pots for winter seed production in a greenhouse near Salinas, California. Six plants of G52 and six plants of G54 were grown to seed maturity and harvested in May of 1998.

F2 seed from these plants were designated as follows:

G52	G52-1 G52-2 G52-3	
G54	G54-1 G54-2 G54-3	G54-4 G54-5 G54-6

Origin and Breeding History

F₂ seed was trialed in the Salinas Valley of California during the summer of 1998 with initial evaluations for Corky root rot susceptibility, bolting, color, and leaf type. The results of the two screening trials were that another generation of selection work and evaluation was needed to better identify resistant root structures. Overall, several lines appeared to have notable segregation and promising levels of desired type.

F₃ Seed was harvested in August of 1998 as follows:

I.D.	Selections	Seed Color
G52-1-	1,2,3,4,5,6,7	all bs
G52-2-	1,2,3,4,5,6,	all bs
G54-1-	1,2,3,4,5,6	all bs
G54-2-	1,2,3,4,5,6,7,8,50	all bs
G54-3-	1,2,3,4,5	all bs
G54-4-	1,2,3,4,5,6,7,8,9,10	all bs
G54-5	1,2,3,4,5,6,7,8,9,10,11,12	all bs

F₃ lines harvested in the fall of 1998 were trialed in the late fall in Yuma, Arizona. The lines were screened for bolt tolerance, desirable dark leaf color, with leaf frill and texture similar to Ventana and North Star. In the desert southwest, Corky Root Rot has not yet been formally reported in any of the fall plantings in lettuce.

In the 1999 seed production crop near Corcoran, California all breeding lines harvested from the 1998 crop were direct seeded for potential harvest in the fall of 1999. Summer trials were planted concurrently in the Salinas Valley to evaluate F_3 lines.

Selection criteria was based on:

Corky Root Rot resistance Slow bolting Dark green color, similar to Two Star

Origin and Breeding History

In the seed crop, early bolting plants, plants with "light" green color; plants with undesirable frill or thinner leaf texture were removed from all lines.

As trials were reviewed in the coastal valleys during June and July and early August, specific lines were identified for further selection work and seed harvest. Lines that did not appear to have potential from trials were destroyed in the seed field.

In 1999, the following lines were harvested:

I.D.	Selections	Seed color	Remarks
G52-1-4-	1,2,3,4,5,Bal	bs	Needs heat, 50 % slow bolt
G52-1-6-	1,2,3,4,5,Bal	bs	,
G52-2-3-	1,2,3,4,5,Bal	bs	Upright, tall stature
G52-2-4-	1,2,3,4,5,Bal	bs	2 colors, slow bolt
G52-2-5-	1,2,3,4,5,Bal	bs	,
G54-1-2-	1,2,3,4,5,Bal	bs	Large frame, excellent color
G54-1-3-	1,2,3,4,5,Bal	bs	Large frame, excellent color
G54-1-4-	1,2,3,4,5,Bal	bs	Tall, segregating color
G54-1-5-	1,2,3,4,5,Bal	bs	No tipburn, reflex involucre
G54-2-7-	1,2,3,4,5,Bal	bs	Segregating color, slow bolt
G54-3-3-	1,2,3,4,5,Bal	bs	Reflex involucre
G54-4-3-	1,2,3,4,5,Bal	bs	early bolters
G54-4-4-	1,2,3	bs	reflex involucre
G54-5-8-	1,2,3,4,5,Bal	bs	
G54-5-11-	1,2,3	bs	
G54-5-12-	Mass	bs	

In the summer of 2000, a key trial in the development of Tehama was evaluated on July 14 near Soledad, California. This trial was planted on May 17, 2000 and evaluated on July 14, 2000. This trial provided an opportunity to evaluate F₄ lines for bolt tolerance, tipburn resistance, and Corky Root resistance under summer harvest conditions. In this trial, several lines scored very high for leaf type intermediate between Two Star and Ventana, and very clean roots as compared to the Corky Root susceptible field varieties Genecorp Green and Two Star. With high cores exhibited in the field varieties, several lines were notably slower in seed stalk development, with a clean, open appearance as opposed to the seed stalk elongation observed in Genecorp Green.

Trial results eliminated thirty-one of the segregating lines in favor of the following five lines that exhibited most promise. These screens narrowed the field to the top ten percent of breeding lines.

Origin and Breeding History

Seed was harvested from the 2000 seed crop as follows:

I.D.	Selections	Seed color
G52-2-3-1	1,2,3,4,5,Bal	bs
G54-1-2-3-	1,2,3,4,5,Bal	bs
G54-1-2-4-	1,2,3,4,5,Bal	bs
G54-2-7-3-	1,2,3,4,5,Bal	bs
G54-2-7-5-	1,2,3,4,5,Bal	bs

Based on the results of trials conducted in 2000, small mass increases of two lines were made in 2001. Additional single plant selections were made from each of the sub-lines and planted in trials to further evaluate for project objectives. The resistance for Corky Root Rot (cor) gene was fixed at the fourth generation, and confirmed at the F₅.

Approximately 25 pounds of experimental seed of the following two lines were grown in 2001:

Trials of the two designated lines were conducted in the desert southwest during the fall of 2001, and the winter and spring of 2002.

Trials were conducted in Yuma, Arizona, Imperial Valley of California, Huron and King City, California. In these trials the G52 line exhibited a high level of heading under cooler than normal growing conditions. Heading was also noted in Two Star, Big Star and other green leaf varieties. G54 remained open, and did not form heads in the winter and spring trials, and was able to produce plants of marketable size with excellent color and texture.

Based on the trial results of 2001 and spring trials of 2002, the G52 line was dropped from the program.

On February 27, 2002, the name "Tehama" was reserved with the Department of Agriculture. (See attached letter).

Origin and Breeding History

The second seed crop of Tehama was produced in the San Joaquin Valley of California near Corcoran in 2002. The crop was uniform to type, and no off-types or variants were noted in this production.

Tehama (Exp. G54-273) was developed using traditional cross pollination techniques, one generation of single seed descent, three generations of single plant selections, and two generations of mass selection to establish a stable bolt tolerant, Corky Root resistant, tipburn resistant, non heading, very dark green thick leaved green leaf lettuce.

Tehama has been observed for two generations of reproduction and during the seed increase period is stable and uniform. No variants were observed.

Exhibit B

Novelty Statement Tehama

Tehama is most similar to Ventana, however, Tehama differs from Ventana in the following characteristics:

The leaf color of Tehama is 143B, whereas, the leaf color of Ventana is 143C based on comparisons made in Yuma, Arizona, December, 2002 using the Royal Horticultural Colour Chart.

The spread of the frame leaves of Tehama is 38.1 cm., whereas, the spread of frame leaves for Ventana is 30.5 cm. (Corcoran, California, June 2002)

Tehama was 62 days to seed stalk elongation during the 2002 seed production season, whereas, Ventana showed seed stalk elongation in 60 days. (Plant date 04-15-02). Note: Genecorp Green was 52 days to first date of seed stalk elongation.

Tehama is resistant to Lettuce Necrotic Stunt Virus, whereas, Ventana is susceptible.

Mature leaf undulation for Tehama is 'strong', whereas, Ventana is 'moderate'.

Butt shape for Tehama is 'rounded', whereas, Ventana is 'flat'

Butt midrib for Tehama is 'prominently raised', whereas, Ventana is 'moderately raised'.

Tehama differs from Two Star in the following characteristics:

Tehama is resistant to Corky Root (cor gene), whereas, Two Star is susceptible. The common strain found in California is identified as CA 1

Tehama leaf color is 143B, whereas, Two Star is 144B based on comparisons made in Yuma, Arizona, December 2002, using the Royal Horticultural Colour Chart.

Tehama was 62 days to seed stalk elongation during the 2002 seed production season, whereas, Two Star showed seed stalk elongation in 58 days. (Plant date 04-15-02)

The spread of the frame leaves for Tehama is 38.1 cm., whereas, the spread of the frame leaves for Two Star is 35.6 cm. (Corcoran, California, June 2002)

U.S. DEFARTMENT OF AGRICULTURE AGRICULTURAL MARKETING SERVICE SCIENCE DIVISION RIFCTIVE DESCRIPTION OF MARIETY

OBJECTIVE DESCRIPTION OF VARIETY LETTUCE Lactuca sativa

	LETTU	ICE <u>Lactuca sativa</u>	•	
NAME OF APPLICANT (S)	aragon Seed, In	100	FOR OFF	ICIAL USE ONLY
ADDRESS (Street and No. or R.F.D. No			· PVPO NUMBER	300078
•	07 Abbott Stree	^+	VARIETY HAME	Tehama
	alinas, Califor		EXPERIMENTAL D	
<u>.</u>	alinas, Califor	. III a _95901	1	- 273
Place numbers in the boxes for the chara spaced plants. Royal Horticultural Socie	cters which best describe this ty or any recognized color sta	variety. Measured data sho andard may be used to dete	ould be the mean of an appropriatemine plant colors.	s number (at least 10) of w
The location of the test area is:	V	Color System	Used: Roval Horticu	ltural Societ
1. PLANT TYPE: (See list of sugges	Yuma, Arizona sted check varieties page 4.)			
01=Cutting/Leal 02=Butterhead 03=Bibb 04=Cos or Roma	(05-Great Lakes 06-Vanguard Gr 07-Imperial Gro	oup 10=Latin up 11=OTH		
2. SEED: COLOR 1=White (Silver Gray)	LIGHT DOR	MANCY	HEAT DORMANCY	
2 2-Black (Gray Brown 3-Brown (Amber)	1=! icht Recu	111	1=Susceptible 2=Not Susceptible	
3. COTYLEDON TO FOURTH LEAF ST	TAGE: NOTE: Provide a co	olar photograph or photocor optimal conditions.	py of the fourth leaf from 20 day	old seedling
3 SHAPE OF FOURTH LE.	2 3	4	5 6	B
1 3 LENGTH/WIDTH INDEX	OF FOURTH LEAF: L/W x	10		
3 APICAL MARGIN: 6 BASAL MARGIN:	1=Entire 2=Creanate/Griawed 3=Finely Dentate	4=Moderately Dentate 5=Coarsely Dentate 6=Incised	7=Lobed 8=OTHER (specify)	
3 UNDULATION:	1-Flat	2=Slight	3=Medium	4≃Marked
GREEN COLOR:	1-Yellow Green 2-Light Green	3=Medium Green 4=Dark Green	5≖Blue Green 6≃Silver Green	7=Gray Green
ANTHOCYANIN: 1 DISTRIBUTION:	1=Absent 2~Margin Only	3=Spotted 4=Throughout	5~OTHER (specify)	
0 CONCENTRATION:	1-Light	2=Moderate	3≃Intense	`.
2 ROLLING:	1-Absent	2=Present		
2 CUPPING:	1=Uncupped	2-Slight	3=Markedly	

2"Apical Margin

3-Lateral Margins

1-None

REFLEXING:

		E LEAVES (observe harvest-mature outer leaves):	20	0300(78
NOT	E: P	Provide color photo of hervest-mature leaves which accurately shows color MARGIN:	and margin characteristics.	9 A A A A	y
evitoriesservo	3	INCISION DEPTH: 1-Absent/Shallow (Dark Green Boston Ideepest panetration of the margin)) 2-Moderate (Vanguard)	3=Deep (Grea	it Lakes 659)
A Complete Williams I and I an	5	INDENTATION: 1=Entire (Dark Green Boston) (finest divisions of the margin) 2=Shallowly Dentate (Great Lakes 65)	3-Deeply Dentate (Great Lakes 659) 4-Crenate (Vanguard)	5-OTHER (. Ventai	pecify) 1 a
on to residence	3	UNDULATION OF THE 1~Absent/Slight (Dark Green Boston)	2=Moderate (Vanguard)	3=Strong (Gre	eat Lakes 659)
ocesso accentration in	4	GREEN COLOR: 1=Very Light Green (Bibb) 2=Light Green (Minetto)	3=Medium Green (Great Lakes) 4=Dark Green (Vanguard)	5=Very Dark (6=OTHER_	Green
and the same of th	,	ANTHOCYANIN (grown at or below 10 C):			
Taken and the best	1	DISTRIBUTION: 1=Absent 2=Margin Only (Big Boston)	3-Spotted (Calif. Cream Butter) 4-Throughout (Prize Head)	5-OTHER (#)	pecify)
	0	CONCENTRATION: 1=Light (Iceberg)	2=Moderate (Prize Head)	3=Intense (Ru	by)
	3	SIZE: 1-Small	2-Medium	3×Large	
SPA missaku Canada Spania	2	GLOSSINESS: 1=Dull (Vanguard)	2=Moderate (Salinas)	3=Glossy (Grea	st Lakes)
SKANNOI INSIGERE	2	BLISTERING: 1=Absent/Slight (Salinas)	2=Moderate (Vanguard)	3=Strong (Prize	: Head)
enterprise	3	LEAF THICKNESS: 1=Thin	2=Intermediate	3=Thick	
The second secon	2	TRICHOMES: 1=Absent (smooth)	2=Present (spiny)		
5. PLAN	T (at	market stage. Choose a comparison variety appropriate for this type.): SPREAD OF FRAME LEAVES:			
3	8	cm This Variety 3 0 cm Ventana		v)	
		HEAD DIAMETER (market trimmed with single cap leaf):		. ,	
		cm This Variety cm	(specify comparison variety	y)	
	5	HEAD SHAPE: 1~Flattened 2~Slightly Flattened	3=Spherical 4=Elongate	5=Non-Heading 6=OTHER	
Substitution of the substi	3	HEAD SIZE CLASS: 1=Small	2-Medium	3=Large	
2	4	HEAD COUNT PER CARTON			
4 9	5	HEAD WEIGHT: g This Variety 4 6 5 g Ventana	(specify comparison variety	9	
	1	HEAD FIRMNESS: 1=Loose 2=Moderate	3=Firm 4=Very Firm		
6. BUTT	(pott	om of market-trimmed head):			
	3	SHAPE: 1-Slightly Concave	2=Flat	3-Rounded	
	3	MIDRIB: 1≈Flaπened (Salinas)	2=Moderately Raised	3=Prominently F	Paised (Great Lakes 659)
	rtem	of market-trimmed head): mm Diameter at base of head			
	의 -	Ratio of head diameter/core diameter			
		Core height from base of head to apex:			
<u> </u>		mm This Variety 3 8 mm Ventana	(specify comparison variety)		
a. BOLTIV	iG (C	Dive First Water Date 4/15/02): NOTE: First Water Date is to germinate. This can and of	he date seed first receives adequate moist ten does equal the planting date.	ŢU r ®	
6	2	Number of days from First Water Date to seed stalk emergence (summer co			
	2 [BOLTING CLASS. 1=Very Slow		-Very Rapid	
1 2 2	>	Height of mature seed stalk: This Variety 1 1 5 cm Ventana	(specify comparison variety)		
FORM LS-4	70-1	BOLTING cont'd, on next pag			Page 2 of 4

Spread of Bolter Plant for who		Ventana (specify con	200300078
BOLTER LEAVES:	1*Straight	Ventana (specify con	πρατμόπ ναπε(γ)
2 MARGIN:	1×Entire	2-Dentate	
3 COLOR:	≻Light Green	2-Medium Green	3-Dark Green
BOLTER HABIT: TERMINAL INFLORESCENCE:	1-Absent	2×Present	
LATERAL SHOOTS:	1=Absent	2=Present	
BASAL SIDE SHOOTS:	1=Absent	2=Present	
. MATURITY fearliness of harvest-mature he			
NOTE: Complete this section for at leas	-		
SEASON Applic. 1 #of days	Check 1/ #of days	CHECK VA	ARIETY 2/
Spring 85	8 5	Two Star	
Summer 6 0	5 8	Shining Star	
Falt 6 1	6 3	North Star	
Winter 94	9 4	Two Star	
Give planting date(s), and location(s):			
Spring 02/05/02	Gonza	les, Ca. R.C. Farms	Williams Ranch
Summer 06/03/02	Gonza	les, Ca. R.C. Farms	Home Ranch
Fall 09/23/02	Wellt	on, Az. Pasquinell	i E. Sassman N.
Winter 12/14/01 L/ First water date to harvest.			Moorhead 20
	2) Fill IN ERBOX VSAI	ety name on the appropriate line.	······································
PRIMARY REGIONS OF ADAP	TION (tested and provi	en adapted): (0=Not tested 1=1	Not Adapted 2*Adapted1
2 Southwest (Colif., Ariz, desert)	2 West Coas	t 2 Northeast	
2 Northcentral	2 Southeast	OTHER	
SEASON: X Spring large Ca. Az	•	x Fall (area Ca. Az. I	71a)
X Summer (area <u>Ca. NJ</u>	, F1a)		\z)
O GREENHOUSE: 0-	Not tested	1=Not Adepted	2=Adapted
2 SOIL TYPE: 1-	Mineral	2~Organic	3 - 80th

FORM LS-470-1 (9-86) Page 3 014

11. DISEASES AND STRESS REACTIONS (0-Not tested; 1-Susceptible; 2-Intermediate; 3-Resistant; 4-Highly resistant; 5 VIRUS FUNGAL/BACTERIAL 1 Big Vein 3. Corky Root Rat (Pythium Root Rat) 10/25/0 1 Lettuce Mossic Downy Mildew (Reces_ O Cucumber Mossic Powdery Mildew 0 Broad Bean Wilt Sclerotinia Rot O Turnip Mousic Bacterial Soft Rot (Pseudomonas spp. & others) 0 Beet Western Yallows Botrytis (Gray Mold) O Lett. Infectious Yellows OTHER __ Other Virus INSECTS PHYSIOLOGICAL/STRESS Cabbage Loopers 3 Tipbum 0 | Salt Root Aphids 5 Heat O Brown Rib (Rib Discoloration, Rib Blight) O Green Peach Aphid 0 Drought OTHER Other Insect 2 Cold POST HARVEST O Pink Rib O Internal Rib Necrosis (Blackheart, Gray Rib, Gray Streak) () Russet Spotting O Brown Stain 0 Rusty Brown Discoloration 12. BIOCHEMICAL OR ELECTROPHORETIC MARKERS:

13. COMMENTS:

SUGGESTED CHECK VARIETIES

	T	Y	ρ	E
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CUTTING/LEAF

2) BUTTERHEAD

3) BIBB

41 COS, OR ROMAINE

GREAT LAKES GROUP

VANGUARD GROUP IMPERIAL GROUP

EASTERN GROUP 81

9) STEM

10) LATIN

CHECK VARIETY

SALAD BOWL DARK GREEN BOSTON 8188 PARRIS ISLAND GREAT LAKES 659-700 VANGUARD VIVA ITHAÇA CELTUCE MATCHLESS

Paragon Seed, Inc.



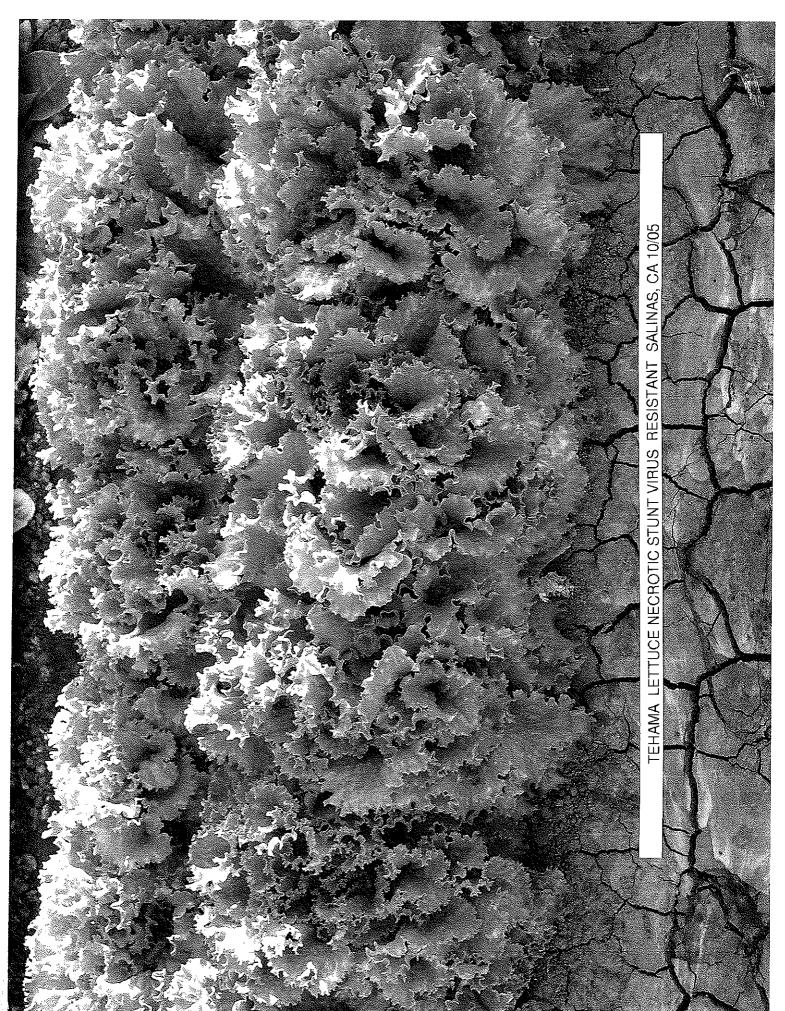


VENTANA

TEHAMA



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PROJECT REPORT CALIFORNIA LETTUCE RESEARCH BOARD

April 1, 2001-March 31, 2002

PROJECT TITLE: THE ETIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT OF A NEW SOIL-BORNE VIRUS DISEASE OF LETTUCE IN CALIFORNIA

W.M. Wintermantel¹, R.C. Grube¹, and S. Koike² USDA-ARS¹ and University of California-Davis² Salinas, California

Lettuce dieback is responsible for losses in Romaine and leaf lettuce production in areas associated with rivers in California and Arizona. Losses vary, ranging from severe in some years to mild in others. Characteristic symptoms include severe stunting, necrosis and dieback of lettuce plants. The disease is caused by isometric viruses in the genus Tombusvirus. Field isolates from lettuce have been identified and characterized as both Tomato bushy stunt virus (TBSV) and the newly described Lettuce necrotic stunt virus (LNSV). Detection of tombusviruses directly from lettuce plants has been difficult. We have now developed a rapid, reliable method for detection of tombusviruses from lettuce tissue. This method decreases the time necessary for confirmation from weeks to a few days. The method involves spin-column purification of total RNA from lettuce, followed by RT-PCR amplification using primers specific to the 3' end of tombusviruses. Tombusviruses are very stable in nature and do not break down easily. As a result, they can survive in soil and water for long periods of time. We have collected soil samples from adjacent lettuce dieback-infested, as well as disease-free fields, and have compared the soil profile of these related soils. Initial studies suggest poor drainage may lead to high salt concentrations, and that these conditions may lead to increased incidence of infection. Adjacent fields with better drainage may have virus, but may not be exposed to conditions leading to disease development. Field testing continues, and research is currently in progress to identify lettuce varieties with resistance to dieback-inducing tombusviruses. Most crisphead varieties are resistant to dieback inducing tombusviruses, and some butterhead and green leaf varieties are available that exhibit resistance. Several romaine, leaf, and stem PI lines have been identified as resistant.

PROJECT REPORT CALIFORNIA LETTUCE RESEARCH BOARD

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PROJECT LEADER:

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Steven T. Koike

Univ. of California Cooperative Extension Salinas, CA 93905 831-759-7356

OBJECTIVES:

- (1) To complete development of a reliable diagnostic assay for *Tomato bushy stunt virus*, the causal agent of lettuce dieback disease, directly from lettuce.
- (2) Begin characterization of epidemiological factors affecting lettuce dieback disease development.
- (3) Continue sequencing of selected portions of the genomes of specific TBSV lettuce isolates and compare these to known sequences.
- (4) Continue to evaluate and identify sources of resistance in field trials involving romaine and leaf lettuce, as well as crisphead varieties.

PROCEDURES AND RESULTS:

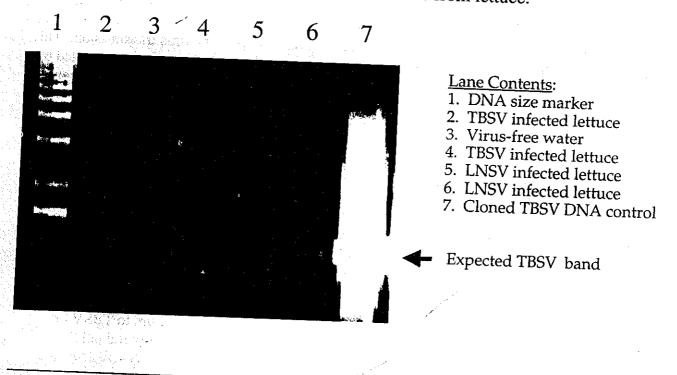
Lettuce dieback is responsible for losses in Romaine and leaf lettuce production in areas associated with rivers in California and Arizona. Losses vary, ranging from severe in some years to mild in others. The disease is tightly linked to the presence of river water, being found commonly in areas where flooding has recently occurred, where river water is used for irrigation of fields, and where dredge is deposited on fields. Characteristic symptoms include severe stunting, necrosis and dieback of lettuce plants. The disease is caused by isometric viruses in the genus Tombusvirus. Field isolates from lettuce have been identified and characterized as both Tomato bushy stunt virus (TBSV) and the newly described Lettuce necrotic stunt virus (LNSV). In addition, Cucumber necrosis virus (CNV) has been identified from diseased fields, however this virus does not appear to contribute significantly to lettuce dieback. Previous studies by our research team have demonstrated that soil fumigation does not prevent virus transmission. This is consistent with a virus that does not require a biological vector. CNV is often transmitted by the soil-borne fungus, Olpidium bornovanus. TBSV, however, is not transmitted by soil-borne fungi. LNSV appears to be more closely related to TBSV than to CNV. Transmissibility of LNSV isolates has not been studied, but transmission of tombusviruses to lettuce does not require a vector, as confirmed by soil fumigation experiments.

Direct detection of tombusviruses from lettuce using RT-PCR

Detection of tombusviruses directly from lettuce plants is difficult, and often detection necessitates passage of the virus to alternate hosts. These alternate hosts serve as indicators for the presence of the virus, by producing characteristic symptoms upon infection. Passaging virus to indicator plants requires considerable time to determine if lettuce was infected with TBSV or other tombusviruses, so this is not a viable method for rapid virus identification. Another method we have successfully developed is immuno-specific electron microscopy. This method requires the availability of an electron microscope, and antiserum to TBSV or LNSV, which we have, however these tools are not readily available to the general public. Serological methods of detection, such as enzyme-linked immunosorbent assays (ELISA) are only partially reliable, since there is considerable diversity among the virus isolates responsible for lettuce dieback disease. Some isolates are detected by an antiserum, while others are not. We have focused our attention recently on development of a reverse transcriptase-polymerase chain reaction (RT-PCR) method for rapid and consistent identification of tombusviruses from infected lettuce plants. Initial attempts with this method were inconsistent, likely because old, diseased lettuce plants often contain inhibitors that interfere with enzyme-based detection methods, such as RT-PCR. We have now developed a rapid, reliable method for detection of tombusviruses from lettuce tissue (Figure 1). This method decreases the time necessary for confirmation from weeks to a few days, and has been highly reliable. The method involves spin-column purification of total RNA from lettuce, followed by "One-step"-PCR amplification using primers specific to the coat protein or 3' end of tombusviruses. The One-step method involves use of a specialized kit that allows for two enzymatic reactions to be performed sequentially in the same tube. The One-step kit is a

trademark of Qiagen Corporation, however, a number of companies provide similar kits which also are effective in detection of lettuce tombusvirus. This method is able to detect diverse isolates from throughout western lettuce growing regions in as short as two days. We will continue to test the RT-PCR-based diagnostic test for tombusvirus detection in lettuce, over the spring and summer to confirm reliability, although currently reliability appears high. The detection method will ultimately be released to extension labs and others expressing interest for use in rapid determination of whether lettuce plants are infected with tombusviruses.

Figure 1. RT-PCR detection of lettuce tombusviruses from lettuce.

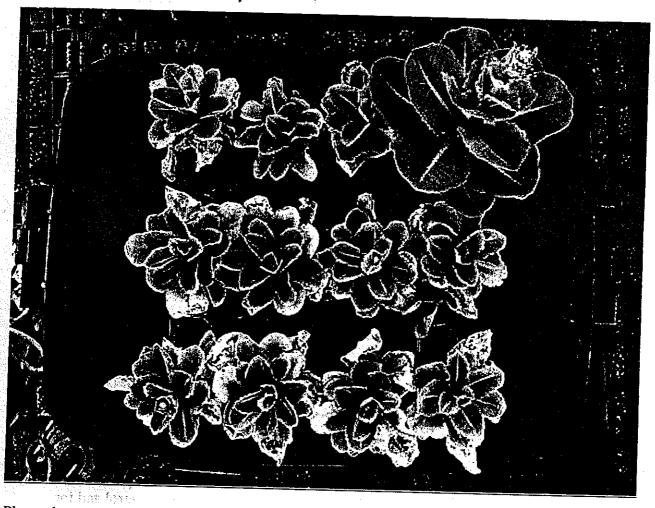


Efficient reproduction of lettuce dieback (TBSV) symptoms under greenhouse conditions.

During the past year we have developed a reproducible method for induction of lettuce dieback disease under greenhouse conditions (Figure 2). This method can produce infection rates of over 90 percent. This is a dramatic improvement over previous methods which required growth chamber conditions and resulted in a maximum of 40 percent infection. The current method should be applicable to both greenhouse and growth chamber, however, evaluation of effectiveness in growth chambers is still in progress. Symptoms of lettuce dieback in the greenhouse remain less dramatic than in the field, but virus can be recovered using the methods described above. Infected plants exhibit the typical veinal necrosis and stunting associated with field symptoms. Seeds are planted in field soil in the greenhouse that has been inoculated with

concentrated plant sap extracted from TBSV-infected *Nicotiana benthamiana* or *N. clevelandii*. One week after planting, additional inoculum is added to the soil, as seedlings begin to emerge. Plants are maintained normally, and symptoms develop within 5-6 weeks post-emergence (Figure 2).

Figure 2. Development of lettuce dieback symptoms on TBSV-infected plants under greenhouse conditions.



Plants shown are romaine lettuce. All plants are infected and symptomatic, with the exception of the plant in the upper right corner. Photo is approximately 8 weeks post-emergence.

Characterization of soil factors leading to increased incidence of lettuce dieback in tombusvirus infested lettuce fields.

Tombusviruses are very stable in nature and do not break down easily. As a result, they can survive in soil and water for long periods of time. We have collected soil samples from adjacent lettuce dieback-infested, as well as disease-free fields, and have compared the soil profile of these samples. Disease free fields adjacent to diseased fields should have similar soil conditions, but may differ in qualities affecting disease incidence. The disease free fields may be infested with tombusviruses, but may not be exposed to soil conditions conducive to disease development. Results of our analysis (Table 1) suggest that poor soil drainage may lead to high salt concentrations and an associated high electrical conductivity of the soil. These conditions may lead to increased incidence of diseased lettuce. Greenhouse and growth chamber experiments are currently being conducted to determine if soil electrical conductivity, anoxic conditions or other factors are primarily responsible for increased disease incidence. We will test dieback disease induction, or if other factors may be responsible.

Characterization of severe tombusvirus isolates and development of resistance to lettuce dieback and lettuce infecting tombusviruses.

Lettuce isolates obtained from fields exhibiting severe outbreaks of lettuce dieback continue to be collected. Once a considerable number of these isolates is obtained, nucleic acid sequence comparisons will be made among the isolates associated with severe outbreaks and isolates associated with occasional or sporadic outbreaks. This will allow us to determine if the severe outbreaks are related to specific tombusvirus isolates, or if environmental factors alone are responsible for increased severity. We gratefully accept samples from severely infested fields for inclusion in this continuing study.

Field testing continues, and research is currently in progress to identify lettuce varieties with resistance to dieback-inducing tombusviruses. Most crisphead varieties are resistant to TBSV, and some butterhead and green leaf varieties are available that exhibit resistance. Several romaine, leaf, and stem PI lines have been identified as resistant. Resistance screens are conducted in the fields of grower-cooperators in the Salinas Valley and in other areas, who have recently experienced losses due to lettuce dieback. Microplots (small isolation plots) have been developed at the USDA-ARS station in Salinas for use in studies on disease control and for selection of resistant lettuce varieties. A more complete discussion of lettuce dieback resistance studies will be presented by the USDA-ARS Lettuce Breeding Program.

Table 1. Results of soil analysis on adjacent Salinas Valley fields with similar soils, but differing by the presence or absence of lettuce dieback disease.

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	. *				meq/L	·		
	SP%	рН_	EC	<u>Ca</u>	Mg	Na_	K	Cl
Field 1D	36	7.3	5.02	31.6	12.5	4.4	1.6	1.9
Field 1H	32	7.7	1.83	10.7	4.0	2.7	0.9	1.2
Field 2D	74	-7.5	2.46	9.8	6.7	7.7	0.4	3.4
Field 2H	74	7.6	1.43	6.6	3.2	4.5	0.4	2.0
Field 3Da	44	7.7	3.7	19.1	10.3	6.8	0.8	4.7
Field 3Db	43	7.7	4.1	19.6	11.6	9.0	0.8	5.4
Field 3H	31	7.5	2.41	11.5	6.5	5.1	0.9	2.6
Field 4H	19	6.0	1.3	5.0	3.2	3.9	0.9	2.7
Mean D	49	7.6	3.82	20.0	10.3	7.0	0.9	3.9
Mean H	39	7.2	1.74	8.5	4.2	4.1	0.8	2.1

Adjacent locations are listed by a single field number. D, Diseased section of field; H, Healthy section of field. Three adjacent field sections were sampled, as indicated, for field 3. Field 4H is the USDA Spence field location, where lettuce dieback has never been observed. SP%, Saturation percentage, is the amount of water necessary to saturate 100g of soil, and is an estimation of soil texture. Soil pH represents the acidity or alkalinity of the soil. EC is the electrical conductivity of the soil and is a measure of the total soluble salt content of the soil. Ca, Calcium; Mg, Magnesium; Na, Sodium; K, potassium; and Cl, Chlorine are soil ion concentrations in milliequivalents/liter. Means indicate the average of all diseased (D) or healthy (H) soils.



g. Lettuce mosaic virus

High resistance to LMV based on the Mild (Mi) and mo gene combination is being introgressed into iceberg and romaine lettuce. Genotypes with high resistance are asymptomatic, or develop mild late appearing symptoms when grown in stressful environments. Susceptible genotypes have severe mottling and stunting, while resistant genotypes have a less severe mottling phenotype. Nine iceberg breeding lines were tested in greenhouse inoculations to identify high resistance breeding lines (Table 3). Six were identified with greater than 75% asymptomatic plants. In resistant Salinas 88 and 3 breeding lines no asymptomatic plants were identified, while only susceptible plants were identified in Salinas. High resistance lines have been crossed to Salinas 88, Vanguard 75, and Clemente. Field plantings of six F₆ breeding lines from 98-169-1 x Vanguard 75 were planted at Spence and a commercial planting to evaluate horticultural characteristics.

h. Lettuce dieback (with W. Wintermantel, S. Koike)

Lettuce dieback is caused by the soilborne tombusviruses. In prior reports, we have shown that all modern iceberg and many green leaf and butter cultivars are resistant, but the majority of red leaf and romaine germplasm is susceptible (Table 4). Romaine breeding lines 01-778M, 01-781M and 01-789M, all developed from PI 491224, were released as resistant germplasm in 2002. In 2003, we found that three commercial romaine cultivars were resistant; the baby romaines Skyway and Defender, and the full size romaine Triple Threat. We also identified a red leaf cultivar with apparent resistance, Ruby Ruffles.

Breeding progress: In 2003, we 1) continued to incorporate resistance from PI491224 into adapted romaine and red leaf types, and 2) begin to transfer resistance from PI 491209 and PI491214 into adapted romaine types. Using resistance from PI491224, F₃ (romaine, red leaf) or F₆ (romaine) progenies were selected in infested and non-infested fields for resistance and type. In romaine type, F₂ and F₃ selections were made and BCF₁ populations were generated using PI491209 and PI491214. Ongoing selection, backcrossing, evaluation and confirmation of resistance and type will continue for all of these materials.

In 2003, we finished evaluating an extensive collection of modern and heirloom cultivars and other genetic materials for susceptibility. This information is being prepared for publication. An updated list of cultivars that appeared resistant in all of our tests is provided (Table 4) Information on additional cultivars will be provided upon request.

We also completed genetic analysis of tombusvirus resistance (see section B, "genetic studies", below). Results from 2003 showed that resistance in iceberg cultivars is caused by a single gene, TvrI, and a position for TvrI on the Iceberg x Saladin molecular map of lettuce was determined. A single gene was also shown to control resistance in PI491224, but it is not yet known whether this gene is the same as TvrI.

Table 3. Lettuce mosaic virus reaction in Salinas, Salinas 88 and 9 breeding lines.

		Numb	er of plants		
Line	susceptible	resistant	asymptomatic	total	Determination -
Salinas	12	0	0	12	susceptible
Salinas 88	0	12	0	12	resistant
98-169-1	0	2	10	12	high resistant
98-170-1	0	0	12	12	high resistant
01-759-1	0	3	9	12	high resistant
01-754-2	0	4 % 2	10	12	high resistant
01-754-3	0	0	12	12	high resistant
02-34-1	0	0	12	12	high resistant
98-174-1	0	12	0	12	resistant
01-772-3	0	12	0	12	resistant
02-44-5	0	12.	0	12	resistant

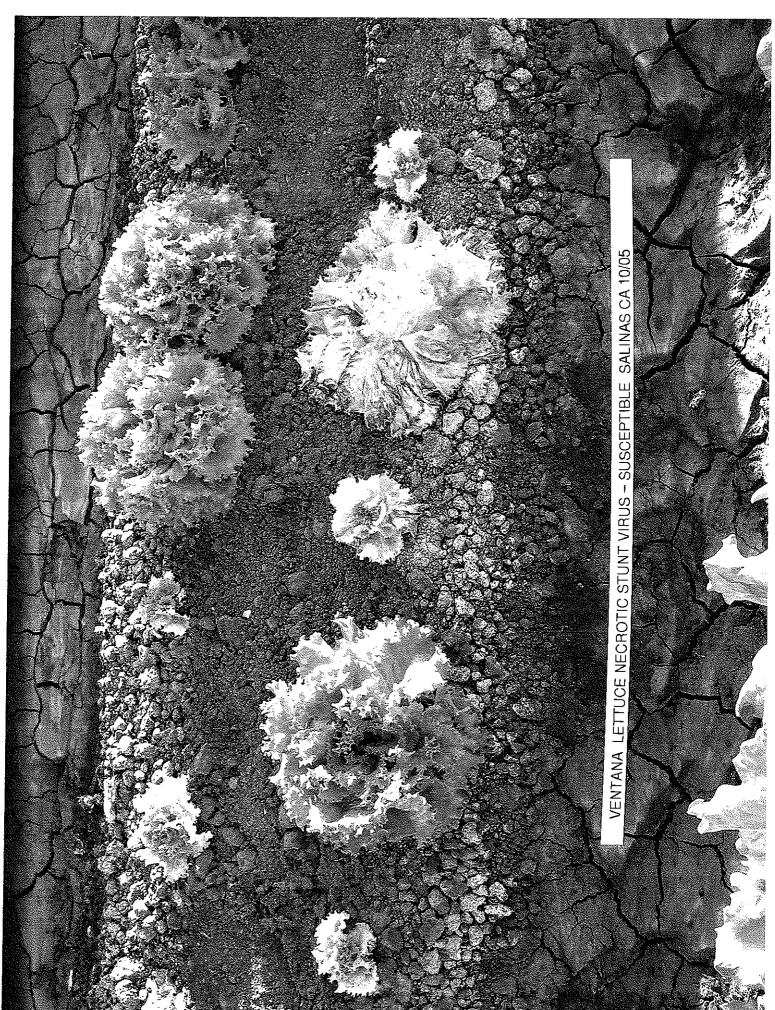
Table 4. Lettuce cultivars that did not show symptoms of lettuce dieback when grown in fields infested with tombusviruses².

Туре	Cultivar	
Butter	Bibb, Big Boston, Buttercrunch, Cinnamon Red, Dark Green Boston, Dynamite, Encanto, Esmerelda, Margarita, Ostinata, Pirat, Pontiac, Tania	
Green leaf	Fanfare, Grand Rapids, Green Valley, Pybas Green, Royal Green, Salad Bowl, Shining Star, Slobolt, Tehama, Two Star, Waldmann's Green	
Crisp/iceberg	All modern cultivars	
Red leaf	Ruby Ruffles	
Romaine	Defender (Shamrock), Skyway (Pybas), Triple Threat (formerly SVR6603, Seminis), PI 491224 , PI 491209, PI 491214	
Other	Balady Banha (stem), Blonde Lente à Monter (heirloom romaine), Cracoviensis (primitive red leaf), Celtuce (stem), Little Gem (latin)	

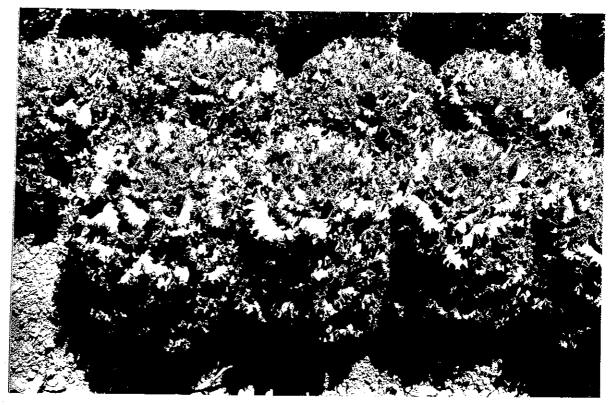
² Plants were direct seeded in fields with prior histories of lettuce dieback. All cultivars listed were free of symptoms in at least two tests in which susceptible control (cultivar Iceberg) showed symptoms.

1

^y This includes ARS breeding lines 01-778M, -789M, and -791M, all derived from PI491224.



June 2002

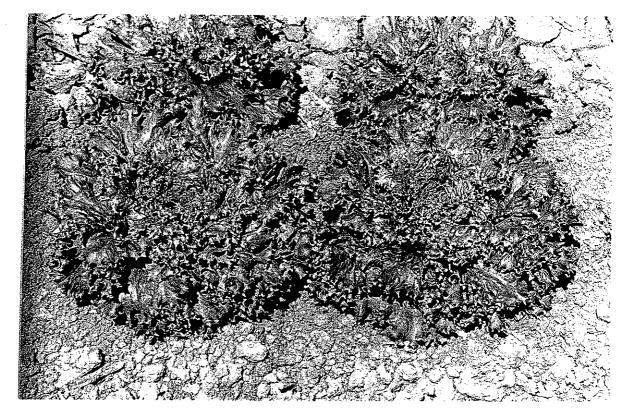


Tehama Green Leaf lettuce ready to bolt

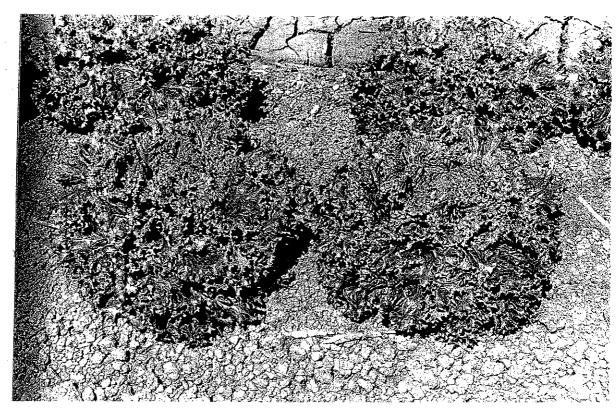


TEHAMA Field production showing uniformity

Paragon Seed, Inc. Corcoran, Ca. 07/98

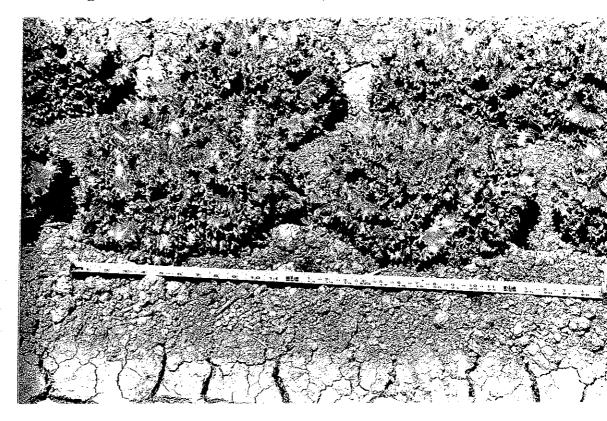


21FR2

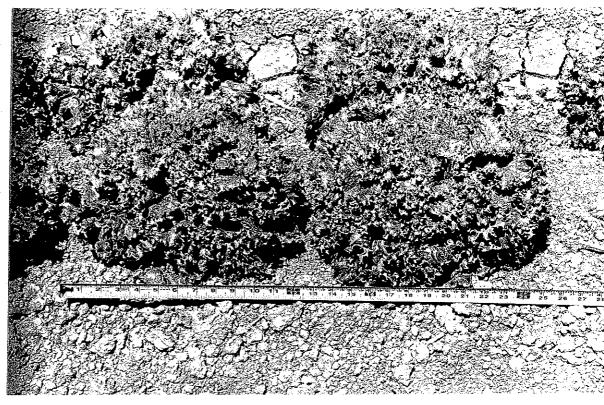


Waldmanns Green

Paragon Seed, Inc. Corcoran, Ca. 07/98

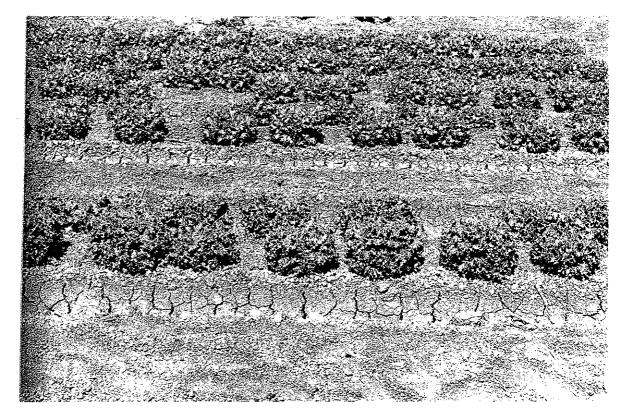


171669-5-4 (spread of frame leaves in seed field) 07-11-98



Waldmanns Green (note seed stem elongation)

Paragon Seed, Inc. Corcoran, Ca. 07/98



171669-5-4 (single bed) 21FR2 (4 beds)



Waldmanns Green (stake) 171669-5-4

200300078

Paragon Seed, Inc. Sargenti Ranch, Chualar, Ca. 07/98



Shining Star left

17166954-97 right

Paragon Seed, Inc. Sargenti Ranch, Chualar, Ca. 07/98



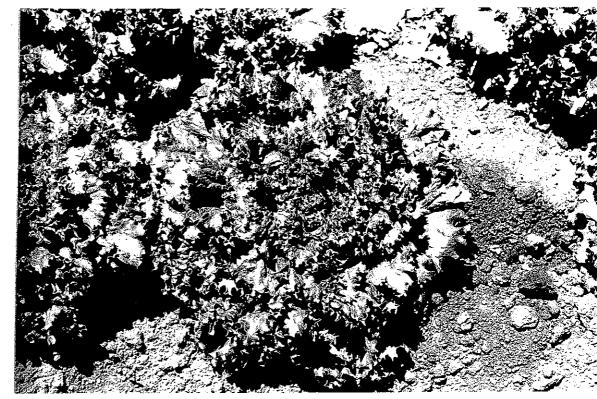
17166954-97 left

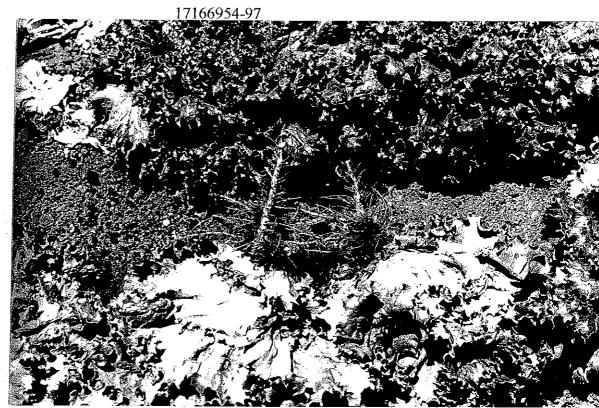
Krypton right



Shining Star Corky root susceptible

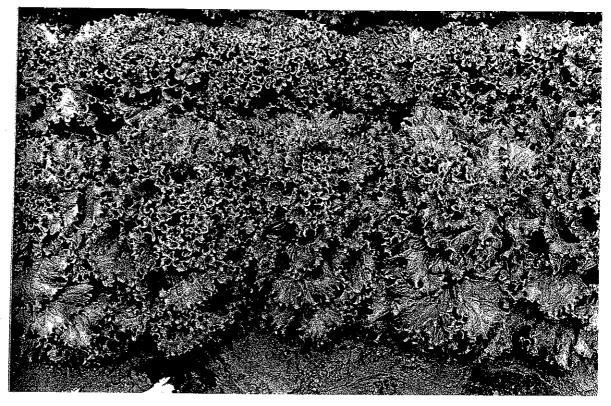
Paragon Seed, Inc. Sargenti Ranch, Chualar, Ca. 07/98





17166954-97

Corky root resistant roots



TEHAMA



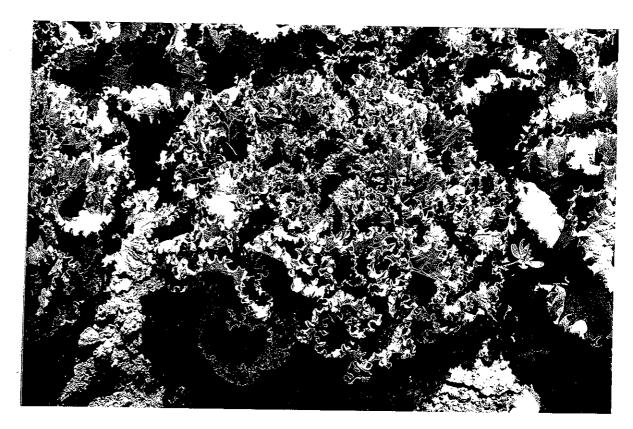
TEHAMA



NORTHSTAR



NORTHSTAR



TEHAMA



BIG STAR



Big Star LEFT Corky Root susceptible

TEHAMA Corky Root Rot Resistant RIGHT



Marin

TEHAMA

GL 47

GL 79



TEHAMA

Paragon Seed, Inc.

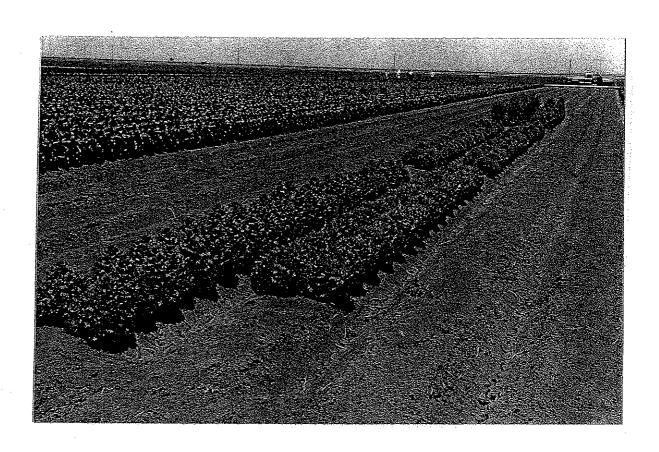
2 STAR

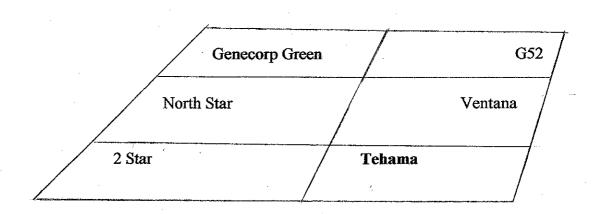
Orsetti Seeds

Paragon Seed, Inc.

June 2002 Corcoran, California

BOLTING COMPARISON OF TEHAMA TO OTHER VARIETIES





June 2002 Corcoran, California

BOLTING COMPARISON OF TEHAMA TO OTHER VARIETIES

TEHAMA	2 Star				
	Ventana No		North	orth Star	
	all which distributed by the community of the department of the de	G 52		Genecorp Green	

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1. NAME OF APPLICANT(S)	TEMPORARY DESIGNATION OR EXPERIMENTAL NUMBER	3. VARIETY NAME	
Paragon Seed, Inc.	G54 - 273	Tehama	
4. ADDRESS (Street and No., or R.F.D. No., City, State, and ZIP, and Country)	5. TELEPHONE (include area code)	6. FAX (include area codé)	
507 Abbott Street	831 - 753-2100	831-753-1470	
Salinas, California	7. PVPO NUMBER		
93901 8. Does the applicant own all rights to the variety? Mark an "X" in appropri	200	300078	
9. Is the prolicest (individual)			
 Is the applicant (individual or company) a U.S. national or U.S. based co If no, give name of country 	mpany?	X) YES NO	
10. Is the applicant the original owner? $\boxed{\underline{\chi}}$ YES $\boxed{}$ N	O If no, please answer one of the fo	ollowing:	
a. If original rights to variety were owned by individual(s), is (are) the original	ginal owner(s) a U.S. national(s)?		
b. If original rights to variety were owned by a company(ies), is(are) the	of if no, give name of country	?	
[] YES [] NO			
11. Additional explanation on ownership (if needed, use reverse for extra spa	GCB):		
LEASE NOTE:	· · · · · · · · · · · · · · · · · · ·		
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P

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- 2. If the rights to the variety are owned by the company which employed the original breeder(s), the company must be U.S. based, owned by nationals of a UPOV member country, or owned by nationals of a country which affords similar protection to nationals of the U.S. for the same genus and species.
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